UFSM

CIÊNCIA e NATURA

ISSN 2179-460X
OPEN ACCESS

Ci. e Nat., Santa Maria, v. 46, e69398, 2024 • https://doi.org/10.5902/2179460X69398
Submissão: 28/06/2023 • Aprovação: 30/06/2023 • Publicação: 15/01/2025

Biology-Genetics

Molecular diagnosis of schistosomiasis mansoni: a literature review

Diagnóstico molecular da esquistossomose mansônica: uma revisão de literatura

Eduardo Henrique Matos Pires[I], Natália Adriely Ribeiro da Silva Costa[I,II], Wheverton Ricardo Correia do Nascimento[II], Walter Lins Barbosa Júnior[I], Elainne Christine de Souza Gomes[I]

[I] Instituto Aggeu Magalhães-Fiocruz, Recife, PE, Brazil
[II] Universidade Federal de Pernambuco, Recife, PE, Brazil

ABSTRACT

Schistosomiasis is a neglected disease caused by trematodes of the genus *Schistosoma*. The molecular diagnosis of the disease is based on the use of molecular biology as a diagnostic tool. This work aimed to carry out an integrative review of the literature, searching for published works that used molecular biology to diagnose human populations affected by schistosomiasis mansoni. Of the selected articles, those that used fecal samples had sensitivity greater than 12% and specificity greater than 29%, while the sensitivity of studies that used urine in general was greater than 5% with specificities greater than 14%. Studies that used serum samples showed sensitivities of 13.9% to 96.3%, with a specificity above 98%. Thus, the study highlights the potential of using molecular biology as an alternative for diagnosing schistosomiasis mansoni in several areas with different levels of endemicity.

Keywords: Schistosomiasis mansoni; Molecular biology; Kato-Katz

RESUMO

A esquistossomose é uma doença negligenciada causada por trematódeos do gênero *Schistosoma*. O diagnóstico molecular da doença se baseia na utilização da biologia molecular como ferramenta de diagnóstico. Este trabalho objetivou realizar uma revisão integrativa da literatura buscando trabalhos publicados que utilizassem a biologia molecular para diagnosticar populações humanas afetadas pela esquistossomose mansônica. Dos artigos selecionados, os que utilizaram amostras de fezes apresentaram sensibilidade maior que 12% e especificidade maior que 29%, enquanto a sensibilidade dos trabalhos que utilizaram urina, de forma geral, foram maiores que 5%, com especificidades maiores

que 14%. Os trabalhos que utilizaram amostras de soro apresentaram sensibilidades de 13,9% a 96,3%, com especificidade acima de 98%. Dessa forma, o estudo destaca a potencialidade da utilização da biologia molecular como alternativa de diagnóstico da esquistossomose mansônica em diversas áreas com diferentes níveis de endemicidade.

Palavras-chave: Esquistossomose mansônica; Biologia molecular; Kato-Katz

1 INTRODUCTION

Schistosomiasis is an infectious disease categorized by the World Health Organization as a neglected disease, which affects an estimated 207 million people distributed in 78 countries (World Health Organization, 2018, 2021). In the Americas, about 1.8 million people are affected by intestinal schistosomiasis caused by *Schistosoma mansoni* and, in Brazil, the prevalence varies from 0.1% to 73.1%, depending on location (Casavechia et al., 2018).

Three population surveys evaluated the prevalence of schistosomiasis in Brazil showing a prevalence of 10% and 6.6% in 1950 and 1977, respectively (Noya et al., 2015; Pellon & Teixeira, 1950). The last survey, carried out between the years 2010 – 2014, estimated the prevalence of the disease at 0.99% (Katz, 2018). Based on the reality of the high national prevalence, between the periods of 1950 and 1977, Katz, Chaves & Pelegrino developed a diagnostic methodology for schistosomiasis mansoni capable of both identifying an infected individual and determining their parasite load, the “Kato-Katz” method (Katz, Chaves & Pelegrino, 1972).

The Kato-Katz method, characterized by its high specificity, is used all over the world and is the gold standard for schistosomiasis diagnosis. However, its sensitivity depends on the parasite load, thus decreasing in patients with low parasite loads. Therefore, new diagnostic alternatives, such as the development of immunodiagnostics and molecular tests, have been studied and developed (Weerakoon et al., 2015). Methodologies that involve the detection of antibodies in the serum of patients are efficient; however, their sensitivity is still lower than those capable of detecting the parasite’s genetic material in biological samples from

patients (Fuss, Mazigo & Muller, 2020). Also, immunological assays for the diagnosis of schistosomiasis have low specificity due to the high number of false-positive diagnoses by cross-reaction (Lin et al., 2008; Xu et al., 2011) alongside the impossibility for detection of active infection, which may result from a host immune response to infections that have already been overcome (Zhou et al., 2011).

Molecular biology techniques have been increasingly studied due to their high sensitivity (Cavalcanti, Cunha & Peralta, 2019) like the polymerase chain reaction (PCR) (Pontes, Dias-neto & Rebello, 2002) and loop-mediated isothermal amplification (LAMP). PCR is a method based on cycles of temperature changes for DNA amplification and has a good sensitivity and specificity in the diagnosis of diseases such as schistosomiasis (Sandoval et al., 2006). LAMP is also efficient in the diagnosis of neglected diseases due to its high sensitivity and its simplicity, requiring only a few reagents and a thermoblock (Abbasi et al., 2010; Notomi et al., 2000).

The LAMP technology is based on DNA amplification using four to six primers under isothermal conditions (Notomi et al., 2000) and is used for the molecular diagnosis of schistosomiasis in humans and vectors, reaching superior sensitivity and specificity results when compared with the Nested-PCR and Kato-Katz (Gandasegui et al., 2018). Furthermore, other techniques derived from conventional PCR were adapted for the diagnosis of this helminthiasis, such as Nested-PCR, Real Time PCR, and PCR-ELISA. Nested-PCR is carried out by amplifying a large DNA segment and then using the product of this reaction for a second amplification. Whereas Real Time PCR does not require electrophoresis to interpret the results, since it uses fluorophores that allow detecting the amplification. These techniques were used to diagnose both schistosomal myelopathy (Brusky et al., 2016), and conventional or classic forms of the disease (do Carmo Magalhães et al., 2020). PCR-ELISA is an immunodetection technique that combines PCR with the ELISA technique and can quantify the PCR product after DNA immobilization, thus is a more sensitive, specific, and faster method than conventional PCR (Sue et al., 2014).

Therefore, this study aimed to review the main molecular diagnostic methods used in the literature to diagnose schistosomiasis mansoni in human biological samples.

2 MATERIALS AND METHODS

This study is based on an integrative review of the subject area of molecular diagnosis for schistosomiasis mansoni.

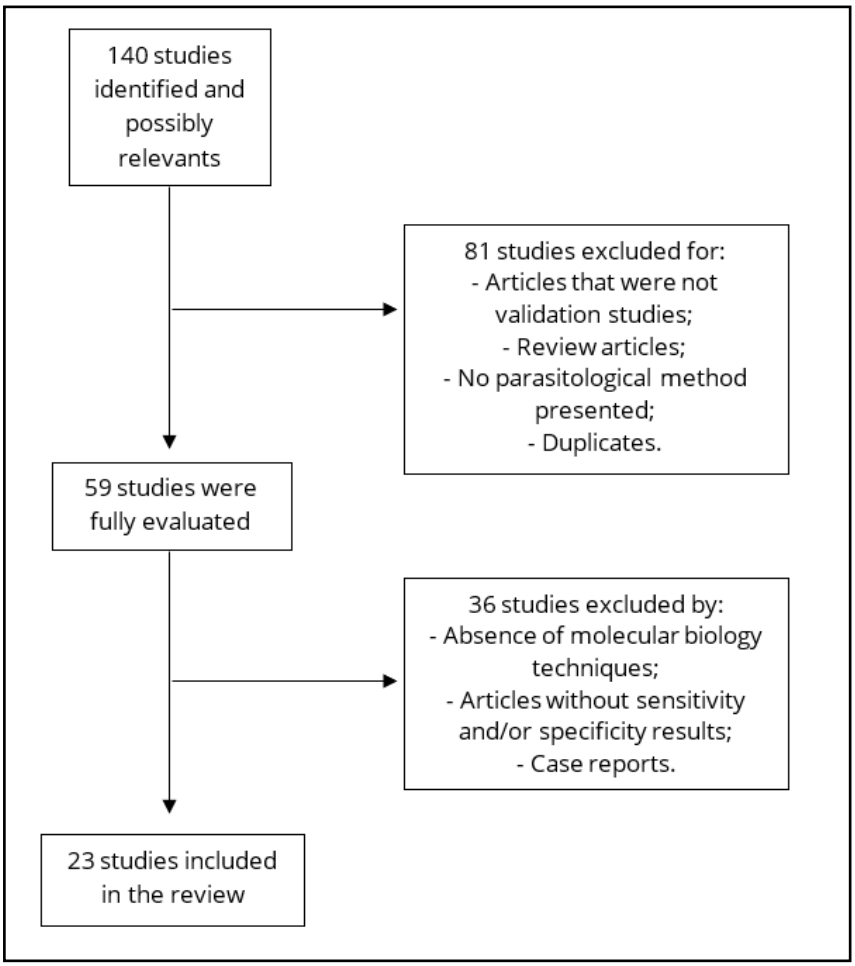
After the choice of the topic, a search was carried out for studies published between the years 2000 to 2023 to restrict the results to articles published since the description of the LAMP technique. Furthermore, only articles in Portuguese and English were analyzed.

The keywords were selected based on the most commonly related to molecular tests to identify *S. mansoni*'s infection: 'LAMP and *Schistosoma mansoni*', 'PCR and *Schistosoma mansoni*', 'Nested PCR and *Schistosoma mansoni*', 'Real time PCR and *Schistosoma mansoni*', 'RFLP PCR and *Schistosoma mansoni*', 'Loop-mediated isothermal amplification and *Schistosoma mansoni*', 'Polymerase chain-reaction and *Schistosoma mansoni*', 'qPCR and *Schistosoma mansoni*', and 'Polymerase chain reaction and *Schistosoma mansoni*'. The keywords were used to search the papers in MEDLINE, PubMed, Science Direct and SciELO bibliographic databases.

Of the articles found in the search, those with the name of the molecular technique and *Schistosoma mansoni* or schistosomiasis in the titles or abstracts were selected and organized in a Microsoft Excel spreadsheet.

The abstracts of the 140 articles that matched the inclusion criteria in the databases were read by the authors to select only those that performed the experimental assays or validation of the techniques. Also, only articles that presented a parasitological method for diagnosis were selected. After this step, repeated articles were excluded, totalizing 59 articles that were fully evaluated (Figure 1).

Figure 1 – Flow diagram for the selection of studies



Source: Authors (2024)

The 59 selected articles were fully read by two reviewers and the following data were extracted for further discussion: population size; sample type; endemicity of the study area; prevalence based on epidemiological data obtained from the Kato-Katz 'gold standard technique'; prevalence based on the evaluated molecular test; sensitivity; specificity; molecular target; extraction method; and a short summary of the article with some keywords. To evaluate the sensitivity of the molecular methods and gold standard technique (Kato-Katz), it was created a health indicator denominated prevalence ratio. The aim of this indicator was to identify the best test for the diagnosis of schistosomiasis by comparing the results of the prevalences found by surveys using molecular and gold standard methods. The prevalence ratio was calculated by

a simple division between the prevalence found by molecular technique (numbered) divided by the results of prevalence found by gold standard (denominator). When the prevalence ratio was <1, the gold standard test had a higher prevalence than the molecular test, nonetheless when the prevalence ratio was >1, the molecular test had a higher prevalence than the gold standard method. After that, articles that did not use molecular biology techniques for the diagnosis of schistosomiasis mansoni in human hosts, articles that lacked sensitivity and/or specificity results and articles that were case reports were excluded, resulting in 23 articles (Chart 1). The results will be presented as: 1) The general overview of all works in each type of sample; 2) Deeper view of each work and its epidemiological results 3) Comparing results by type of sample and molecular reaction.

Chart 1 – References included in the review

(continue...)

ID	Study Location	Year of publication	References
1	Madagascar	2014	Schwarz, N. G., Rakotozandrindrainy, R., Heriniaina, J. N., Randriamampionona, N., Hahn, A., Hogan, B., ... & Hagen, R. M. (2014). *Schistosoma mansoni* in schoolchildren in a Madagascan highland school assessed by PCR and sedimentation microscopy and Bayesian estimation of sensitivities and specificities. *Acta tropica*, *134*, 89-94.
2	Brazil	2020	do Carmo Magalhães, F., Resende, S. D., Senra, C., Graeff-Teixeira, C., Enk, M. J., Coelho, P. M. Z., ... & Carneiro, M. (2020). Accuracy of real-time polymerase chain reaction to detect *Schistosoma mansoni*–infected individuals from an endemic area with low parasite loads. *Parasitology*, *147*(10), 1140-1148.
3	Brazil	2014	Espírito-Santo, M. C. C., Alvarado-Mora, M. V., Dias-Neto, E., Botelho-Lima, L. S., Moreira, J. P., Amorim, M., ... & Gryschek, R. C. B. (2014). Evaluation of real-time PCR assay to detect *Schistosoma mansoni* infections in a low endemic setting. *BMC Infectious Diseases*, *14*, 1-10.
4	Mozambique	2017	Meurs, L., Polderman, A. M., Vinkeles Melchers, N. V., Brienen, E. A., Verweij, J. J., Groosjohan, B., ... & van Lieshout, L. (2017). Diagnosing polyparasitism in a high-prevalence setting in Beira, Mozambique: detection of intestinal parasites in fecal samples by microscopy and real-time PCR. *PLoS neglected tropical diseases*, *11*(1), e0005310.
5	Tanzania	2020	Fuss, A., Deogratias Mazigo, H., & Mueller, A. (2020). Evaluation of serum-based real-time PCR to detect *Schistosoma mansoni* infection before and after treatment. *Infectious Diseases of Poverty*, *9*(03), 114-120.

Chart 1 – References included in the review

(continue...)

ID	Study Location	Year of publication	References
6	Tanzania	2018	Fuss, A., Mazigo, H. D., Tappe, D., Kasang, C., & Mueller, A. (2018). Comparison of sensitivity and specificity of three diagnostic tests to detect *Schistosoma mansoni* infections in school children in Mwanza region, Tanzania. *PloS one*, *13*(8), e0202499
7	Ethiopia	2015	Schunk, M., Kebede Mekonnen, S., Wondafrash, B., Mengele, C., Fleischmann, E., Herbinger, K. H., ... & Zeynudin, A. (2015). Use of occult blood detection cards for real-time PCR-based diagnosis of *Schistosoma mansoni* infection. *PLoS One*, *10*(9), e0137730.
8	Ghana	2014	Lodh, N., Naples, J. M., Bosompem, K. M., Quartey, J., & Shiff, C. J. (2014). Detection of parasite-specific DNA in urine sediment obtained by filtration differentiates between single and mixed infections of *Schistosoma mansoni* and S. haematobium from endemic areas in Ghana. *PLoS One*, *9*(3), e91144.
9	Zambia	2013	Lodh, N. (2013). Diagnosis of *Schistosoma mansoni* without the Stool: Comparison of Three Diagnostic Tests to Detect Schistosoma mansoni Infection from Filtered Urine in Zambia (vol 89, pg 46, 2013). *American Journal of Tropical Medicine and Hygiene*, *89*(3), 608-608.
10	Ghana	2020	Anyan, W. K., Pulkkila, B. R., Dyra, C. E., Price, M., Naples, J. M., Quartey, J. K., ... & Lodh, N. (2020). Assessment of dual schistosome infection prevalence from urine in an endemic community of Ghana by molecular diagnostic approach. *Parasite epidemiology and control*, *9*, e00130.
11	Brazil	2003	Pontes, L. A., Oliveira, M. C., Katz, N., Dias-Neto, E., & Rabello, A. N. A. (2003). Comparison of a polymerase chain reaction and the Kato-Katz technique for diagnosing infection with *Schistosoma mansoni*. *The American journal of tropical medicine and hygiene*, *68*(6), 652-656.
12	Zambia	2017	Hessler, M. J., Cyrs, A., Krenzke, S. C., Mahmoud, E. S., Sikasunge, C., Mwansa, J., & Lodh, N. (2017). Detection of duo-schistosome infection from filtered urine samples from school children in Zambia after MDA. *PLoS One*, *12*(12), e0189400.
13	Brazil	2012	Carvalho, G. C. D., Marques, L. H. D. S., Gomes, L. I., Rabello, A., Ribeiro, L. C., Scopel, K. K. G., ... & Abramo, C. (2012). Polymerase chain reaction for the evaluation of *Schistosoma mansoni* infection in two low endemicity areas of Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz*, *107*, 899-902.
14	Brazil	2012	Enk, M. J., Oliveira e Silva, G., & Rodrigues, N. B. (2012). Diagnostic accuracy and applicability of a PCR system for the detection of *Schistosoma mansoni* DNA in human urine samples from an endemic area. *PloS one*, *7*(6), e38947.
15	Venezuela	2020	Ferrer, E., Villegas, B., Mughini-Gras, L., Hernández, D., Jiménez, V., Catalano, E., & Incani, R. N. (2020). Diagnostic performance of parasitological, immunological and molecular tests for the diagnosis of *Schistosoma mansoni* infection in a community of low transmission in Venezuela. *Acta tropica*, *204*, 105360.

Chart 1 – References included in the review

(conclusion)

ID	Study Location	Year of publication	References
16	Brazil	2018	Senra, C., Gomes, L. I., Siqueira, L. M. V., Coelho, P. M. Z., Rabello, A., & Oliveira, E. (2018). Development of a laboratorial platform for diagnosis of schistosomiasis mansoni by PCR-ELISA. *BMC Research Notes*, *11*, 1-5.
17	Brazil	2018	Gandasegui, J., Fernández-Soto, P., Muro, A., Simões Barbosa, C., Lopes de Melo, F., Loyo, R., & de Souza Gomes, E. C. (2018). A field survey using LAMP assay for detection of *Schistosoma mansoni* in a low-transmission area of schistosomiasis in Umbuzeiro, Brazil: Assessment in human and snail samples. *PLoS Neglected Tropical Diseases*, *12*(3), e0006314.
18	Zambia	2019	Price, M., Cyrs, A., Sikasunge, C. S., Mwansa, J., & Lodh, N. (2019). Testing the infection prevalence of *Schistosoma mansoni* after mass drug administration by comparing sensitivity and specificity of species-specific repeat fragment amplification by PCR and loop-mediated isothermal amplification. *The American Journal of Tropical Medicine and Hygiene*, *101*(1), 78.
19	Switzerland	2022	Hoekstra, P. T., Chernet, A., de Dood, C. J., Brienen, E. A., Corstjens, P. L., Labhardt, N. D., ... & van Lieshout, L. (2022). Sensitive diagnosis and post-treatment follow-up of *Schistosoma mansoni* infections in asymptomatic Eritrean refugees by circulating anodic antigen detection and polymerase chain reaction. *The American journal of tropical medicine and hygiene*, *106*(4), 1240.
20	Democratic Republic of the Congo	2022	Hoekstra, P. T., Madinga, J., Lutumba, P., van Grootveld, R., Brienen, E. A., Corstjens, P. L., ... & van Lieshout, L. (2022). Diagnosis of schistosomiasis without a microscope: evaluating circulating antigen (CCA, CAA) and DNA detection methods on banked samples of a community-based survey from DR congo. *Tropical Medicine and Infectious Disease*, *7*(10), 315.
21	Canada	2023	Lau, R., Makhani, L., Omoruna, O., Lecce, C., Shao, E., Cunanan, M., ... & Boggild, A. K. (2023). Performance characteristics of diagnostic assays for schistosomiasis in Ontario, Canada. *Therapeutic Advances in Infectious Disease*, *10*, 20499361231173843.
22	Brazil	2022	de Souza Gomes, E. C., Júnior, W. L. B., & de Melo, F. L. (2022). Evaluation of SmITS1-LAMP performance to diagnosis schistosomiasis in human stool samples from an endemic area in Brazil. *Experimental Parasitology*, *242*, 108389.
23	Brazil	2022	Mesquita, S. G., Caldeira, R. L., Favre, T. C., Massara, C. L., Beck, L. C. N. H., Simões, T. C., ... & Fonseca, C. T. (2022). Assessment of the accuracy of 11 different diagnostic tests for the detection of Schistosomiasis mansoni in individuals from a Brazilian area of low endemicity using latent class analysis. *Frontiers in Microbiology*, *13*, 1048457.

Source: Authors (2024)

3 RESULTS

Of the 23 articles included in this study (Chart 1), 15 used stool samples, 10 urine samples, and 3 serum samples. Of these, 5 evaluated more than one type of biological sample. The articles that used stool samples in their molecular assays showed sensitivity above 12% and specificity above 29.55%. Two studies (References 4, 7) lacked molecular specificity data. The techniques evaluated for this type of sample were qPCR or Real Time-PCR, conventional PCR, PCR-ELISA, and LAMP. Eight articles presented the sensitivity calculated for the gold standard method, which was always a parasitological method associated or not with another diagnostic method. The sensitivity of the parasitological methods was lower than that of the molecular methods evaluated in most of these works, except for the reference 11. A total of 13 works (Reference 1,2,3,6,11,13,15,16,17, 19, 20, 22, 23) presented the calculated specificity for molecular methods and 7 (References 1,2,6,13,15, 20, 23) for parasitological methods.

Regarding the DNA extraction methods used by the studies with stool samples, 9 studies used extraction kits: QIAamp DNA Stool Mini Kit (5); QIAamp Spin Columns/Mini Kit (1); QIAamp Stool Kit (1); QIAamp DNA-easy (2); rapid one-step extraction (ROSE) method (2), one of which was modified; and the phenol-chloroform method (3), one of which was also modified; QIAamp Fast DNA Stool Mini Kit (1); QIAamp PowerFecal Pro DNA Kit (1); One article lacked the method used (reference 16) (Chart 2).

Of the 15 studies with stool samples, 7 selected only the Sm1-7 region as a molecular target, 6 used the ITS2 region, 1 used the mitochondrial minisatellite region of *S. mansoni* and 1 used Sm1-7 and MIT both.

Among those with stool samples, two of them analyzed the sensitivity and specificity of the molecular test using Latent Class Analysis (LCA), which consists of an approach to latent variables, meaning they are not directly observable, which can be quantitative or categorical. This analysis aimed to, based on a set of categorical variables related to the latent variable of interest, identify mutually exclusive subgroups of individuals (Mastella, 2015). Thus, study 6 considered the true *S. mansoni* infection

status as a latent variable with two categories: 'infected' and 'uninfected'. From this, it analyzed sensitivity and specificity relating the true class of the disease and the results observed. While study 23 used LCA to evaluate the probability of each individual involved in the research being classified as 'case'.

The highest sensitivity found for stool samples was 98.70%, obtained by references 6 and 13 in a qPCR and conventional PCR assay, respectively, in which both used the QIamp DNA stool mini kit and the Sm1-7 region as a molecular target. The specificity of those tests was 81.20 and 100%, respectively. Study 6 and 23 calculated the sensitivity and specificity values both using Kato-Katz as the gold standard method and using latent class analysis, whose results mentioned refer to this analysis. Studies 13 and 15 showed the highest specificity, 100%, in a conventional PCR assay. Study 13 was the most sensitive and specific among the studies that used stool samples for the diagnosis of schistosomiasis. The studies with greater sensitivity and specificity for the qPCR, PCR-ELISA, and LAMP reactions were studies 23 (sensitivity: 96%; specificity: 94%), 16 (sensitivity: 97.40%; specificity: 91.10%), and 17 (sensitivity: 92.86%; specificity: 80.11%), respectively.

A total of 10 studies (References 1,2,3,6,11,13,15,16,17, 23) calculated the prevalence based on the molecular methods and the parasitological methods. Prevalence ratios ranged from 0.35x (Reference 23) to 10.66x (Reference 1). Almost all the studies that presented this data had a higher prevalence by the molecular diagnosis than by the gold standard method, resulting in a ratio greater than 1. However, one study had a higher prevalence by the gold standard than by the molecular diagnosis (Reference 23) obtaining a prevalence ratio below 1.

Of the studies that analyzed stool samples, 8 calculated the positive and negative predictive values (PPV and NPV) (References 2,3,11,13,15,17, 22 and 23), and the study 2 showed only the positive predictive value, for the molecular tests. The highest PPV was 100%, showed by articles 13 and 15, whereas the highest NPV was 99.8% (Reference 3). The highest PPV and NPV in the same work were 100% and 99.30% in study 13 (Chart 2).

Chart 2 – Variables detailed in the articles that used stool samples in their molecular assays

(continue...)

ID	1	2	3	4	6	7	11	13
Molecular Method	Real Time PCR	Real Time PCR	Real Time PCR		Real Time PCR	Real Time PCR	PCR	PCR
Molecular sensitivity	95.3%	91.4%	80%	97%	99.5% or **LCA:** 98.7%	**PCR1:** 94.30% or **PCR2:** 91,40%	96.70%	98.70%
Parasitologic Sensitivity (gold standard)	19.3%	**KK 2 slides:** 36.0%; **KK 6 slides:** 59.2%	-	12%	LCA: 89.7%	-	100%	20.80%
Molecular Specificity	94.9%	80.2%	92%	-	29.55% or **LCA:** 81.2%	-	88%	100%
Parasitologic Specificity (gold standard)	98.5%	**KK 2 slides:** 100%; **KK 6 slides:** 95.7%	-	-	LCA: 72.8%	-	-	100%
Gold standard prevalence	15.2%	20.4%	0.9%	10.9%	85.2%	63.60%	30.90%	7.30%
Molecular prevalence	77.1% or 80.2%	54.4%	9.6%	-	92.9%	-	38.10%	34.70%
Prevalence ratio	10.66x	2.6x	5.07x	-	1.09x	-	1.23x	4.75x
PPV/NPV	-	PPV= 81.2% NPV= -	PPV= 8% NPV= 99.8%	-	-	-	PPV=78.4% NPV=98.30%	PPV=100% NPV= 99.30%
Molecular Target	*ITS2*	*Sm1-7*	*Sm1-7*	*ITS2*	*Sm1-7*	*ITS2*	*Sm1-7*	*Sm1-7*
Extraction Method	QIAamp®DNA Stool Mini Kit	QIAamp®DNA StoolMiniKi	RapidOne--StepEx-traction (ROSE)	QIAampS-pinColumns/ MiniKit	QIAamp DNA Stool Mini Kit	QIAamp Stool Kit	ROSE Modi-fied	QIAamp DNA Stool Mini Kit

Chart 2 – Variables detailed in the articles that used stool samples in their molecular assays

(conclusion)

ID	15	16	17	19	20	22	23
Molecular Method	PCR	PCR-ELISA	LAMP	Real Time PCR	Real Time PCR	LAMP	LAMP; Real time PCR; PCR--ELISA
Molecular sensitivity	69.57%	97.40%	92.86%	56%	39%	12%	**LAMP:** 72% or **Real Time PCR:** 96% or **PCR-ELISA:** 96%
Parasitologic Sensitivity (gold standard)	47.83%	-	-	-	-	-	38%
Molecular Specificity	100%	**PCR-ELISA1:** 91.1% or **PCR-ELISA2:** 85.1%	80.11%	98%	100%	93%	**LAMP:** 75% or **Real Time PCR:** 94% or **PCR-ELISA:** 92%
Parasitologic Specificity (gold standard)	100%	-	-	-	100%	-	99%
Gold standard prevalence	17.10%	18,4%	3.04%	-	-	-	38%
Molecular prevalence	21.60%	**PCR-ELISA1:** 25.2% or **PCR-ELISA2:** 30.10%	30.24%	-	-	-	13.4%
Prevalence ratio	1.2x	1.4x or 1.67x	9.94x	-	-	-	0.35x
PPV/NPV	PPV=100% NPV= 84.54%	-	PPV= 26% NPV= 99.33%	-	-	PPV=59% NPV= 57%	**LAMP:** PPV=28% NPV= 95% or **Real Time PCR:** PPV=68% NPV= 99% or **PCR-ELISA:** PPV=61% NPV= 99%
Molecular Target	*Sm1-7*	*Sm1-7*	minisate-llite	*ITS2*	*ITS2*	*ITS2*	**LAMP:** *MIT;* **Real time PCR and PCR-ELISA:** *Sm1-7*
Extraction Method	Modified Phenol--Chloroform	-	Phenol--Chloro-form	QIAamp DNA-easy	QIAamp DNA-easy	Phenol-Chlo-roform	QIAamp DNA Stool Mini Ki + QIAamp Fast DNA Stool Mini Kit + QIAamp PowerFecal Pro DNA Kit

Source: Authorship (2024)

*Depends on the number of slides used in the KK

**With latent class analysis or not

The molecular sensitivity of molecular methods that used urine for the diagnosis of *S. mansoni* infection was, in general, higher than 5%, while the specificity was higher than 14%. All studies showed both the sensitivity and specificity of the molecular tests. Molecular assays using this type of sample were: real time-PCR, conventional PCR, and LAMP. The sensitivity of the parasitological method was higher than 38%, except for work 12, in which its value was 11% for the Kato-Katz method (Chat 3).

Regarding the extraction method used for the studies with urine, 3 studies used the QIAmp DNA blood mini kit, 1 study used QIAamp DNA-easy and 5 used different methods, those were the QIAamp circulating Nucleic Acid Kit, QIAamp DNA mini kit, QIAmp DNeasy Blood and Tissue Kit, Salting out and resin DNA extraction method, and Chelex 100 resin. Also, a study compared two extraction methods, the QIAmp DNeasy Blood and Tissue Kit and LAMP-PURE extraction kit. Regarding the molecular targets, all studies used the Sm1-7 region, except for study 8 and 20, which used the ITS2 region and study 23 that used the MIT and Sm1-7 region.

The highest sensitivity obtained by molecular tests was 100% in studies 9,10,12,14, and 18. These same studies showed specificities of 100%, 82.60%, 100%, 91.20%, and 100%, respectively. Studies 8 and 15 showed the highest specificities,100%, alongside studies 9, 10, 12 and 18, already mentioned. Studies 9 and 12 showed the best sensitivity and specificity values using conventional PCR as a diagnostic method and the QIAamp DNA mini kit and QIAmp DNeasy Blood and Tissue Kit as extraction method, respectively. Study 18, which used the LAMP technique, obtained sensitivity values reaching 100%, when using the QIAmp DNeasy Blood and Tissue Kit. Whereas the studies that used qPCR, showed a sensitivity of 13% (Reference 23), 5% (Reference 20) and 33.30% (Reference 5). A total of 6 studies (References 5,8,9,12,15, 23) showed the sensitivity data for the parasitological method used; study 15 obtaining the highest one, 47.83%. Whereas 7 studies (References 5,8,9,12,15,20,23) showed the specificity of the gold method, which was 99% (Reference 23) and 100% (References 5,8,9,12, 15, 20) for the Kato-Katz method.

Seven studies used urine samples to analyze the prevalence ratio for both forms of diagnosis, molecular and parasitological, and studies 8, 9, 12 and 14 obtained a higher prevalence by the molecular method than the parasitological with a ratio prevalences greater than 1, reaching 9.75x. Studies 5, 15 and 23 obtained a higher prevalence by the parasitic method than by the molecular ones, with values of 0.35 to 0.89 times lower for the molecular method.

Eight studies also showed the PPV and NPV, and the study 5 showed only the NPV. Studies 8,9,12,15, and 18 showed the highest PPVs, which was 100%. Studies 9,12,14, and 18 showed the highest NPVs, also 100%. Studies 9 and 12 obtained the highest PPVs and NPVs (100%) for molecular tests in urine samples (Chart 3).

Chart 3 – Variables detailed in the articles that used urine samples in their molecular assays

(continue...)

ID	Molecular Method	Molecular sensitivity	Parasitologic Sensitivity (gold standard)	Molecular Specificity	Parasitologic Specificity (gold standard)	Gold standard prevalence	Molecular prevalence	Prevalence ratio	PPV/NPV	Molecular Target	Extraction Method
5	Real Time PCR	33.3%	44.4%	100%	100%	34.3%	30.6%	0.89x	PPV= - NPV= 28%	*Sm1–7*	QIAamp Circulating Nucleic Acid Kit
8	PCR	99%	66%	100%	100%	66%	86%	1.30x	PPV= 100% NPV=92%	*ITS2*	QIAmpDNA Blood Mini Kit

Chart 3 – Variables detailed in the articles that used urine samples in their molecular assays

(continue...)

ID	18	15	14	12	10	9
Molecular Method	LAMP, PCR	PCR	PCR	PCR	PCR	PCR
Molecular sensitivity	**PCR:** 83% or 79% **LAMP:** 100% or 88%	8.70%	100%	100%	100%	100%
Parasitologic Sensitivity (gold standard)	-	47.83%	-	11%	-	57%
Molecular Specificity	**PCR:** 100% or 31% **LAMP:** 100% or 14%	100%	91.20%	100%	82.60%	100%
Parasitologic Specificity (gold standard)	-	100%	-	100%	-	100%
Gold standard prevalence	-	17.10%	35.57%	8%	-	51%
Molecular prevalence	**PCR:** 94% or 88% **LAMP:** 94% or 94%	6.20%	41.24%	78%	46.60%	89%
Prevalence ratio	-	0.36x	1.15x	9.75x	-	1.74x
PPV/NPV	**PCR:** PPV= 100% or 90% NPV= 28% or 16% **LAMP:** PPV= 100% or 94% NPV= 100% or 7%	PPV= 100% NPV=68.18%	PPV= 86.25% NPV= 100%	PPV=100% NPV=100%	-	PPV=100% NPV=100%
Molecular Target	*Sm1-7*	*Sm1-7*	*Sm1-7*	*Sm1-7*	*Sm1-7*	*Sm1-7*
Extraction Method	QIAmp DNeasy Blood and Tissue Kit and LAMP-PURE extraction kit	Chelex 100 resin	Salting out and resin	QIAmp DNeasy Blood and Tissue Kit	QIAmpDNA Blood Mini Kit	QIAamp DNA mini kit

Chart 3 – Variables detailed in the articles that used urine samples in their molecular assays

(conclusion)

ID	20	23
Molecular Method	Real Time PCR	LAMP; PCR; Real Time PCR
Molecular sensitivity	5%	**LAMP:** 19% or **PCR:** 6% or **Real Time PCR:** 13%
Parasitologic Sensitivity (gold standard)	-	38%
Molecular Specificity	100%	**LAMP:** 77% or **PCR:** 94% or **Real Time PCR:** 96%
Parasitologic Specificity (gold standard)	100%	99%
Gold standard prevalence	-	38%
Molecular prevalence	-	13.4%
Prevalence ratio	-	0,35x
PPV/NPV	-	**LAMP:** PPV= 10% NPV=88% or **PCR:** PPV= 12% NPV=88% or **Real Time PCR:** PPV= 29% NPV=89%
Molecular Target	*ITS2*	**LAMP**: *MIT*; **PCR and Real Time PCR:** *Sm1-7*
Extraction Method	QIAamp DNA-easy	QIAamp DNA Blood Mini Kit

Source: Authorship (2024)

For serum samples, the molecular sensitivities were from 13,9% to 96.3% (References 3,5, 21), whereas the molecular specificity values are above 98%. The molecular technique used for this type of sample was only real time-PCR (Chart 4).

The studies with serum used as extraction methods: RapidOne-StepExtraction (ROSE), QIAamp Circulating Nucleic Acid Kit, Chelex 100 resin and Qiagen DNA Mini Kit. As a molecular target, all studies used the Sm1-7 region.

Study 5 showed the highest molecular sensitivity and specificity for serum samples, obtaining a sensitivity of 96.3%, in patients with positive microscopy, and specificity of 100% with a qPCR and QIAamp Circulating Nucleic Acid Kit extraction

method. The sensitivity of parasitological methods of the researches that used serum samples was below 49%.

Two studies (References 3 and 5) that calculated the prevalence by both methods obtained a higher prevalence by the molecular method than by the parasitological method used, reaching a prevalence ratio greater than 1.

Study 3 showed the values of PPV and NPV, 12.5% and 99.30%, respectively. While study 21 showed a PPV value of 100% for qPCR 1 and 2 and a NPV of 26.9 for qPCR 1 and 23.9 for qPCR 2. Study 5 only showed the NPV value for its diagnosis, which was 88.90% (Chart 4).

Chart 4 – Variables detailed in the articles that used serum samples in their molecular assays

ID	Molecular Method	Molecular sensitivity	Parasitologic Sensitivity (gold standard)	Molecular Specificity	Parasitologic Specificity (gold standard)	Gold standard prevalence	Molecular prevalence	Prevalence ratio	PPV/NPV	Molecular Target	Extraction Method
3	Real Time PCR	20%	-	98.8%	-	0.9%	1.4%	1.55x	PPV= 12.5% NPV= 99.3%	*Sm1–7*	RapidOne-StepExtraction (ROSE)
5	Real Time PCR	96.3%	44.4%	100%	100%	34.3%	75.0%	2.18x	PPV= - NPV= 88.9%	*Sm1–7*	QIAampCirculating NucleicAcidKit
21	Real Time PCR	**PCR1:** 27.8% or **PCR2:** 13.9%	-	100%	-	-	-	-	**PCR1:** PPV= 100% NPV= 26.9% or **PCR2:** PPV= 100% NPV= 23.6%	*Sm1-7*	Qiagen DNA Mini Kit

Source: Authorship (2024)

4 DISCUSSION

Schistosomiasis mansoni is a neglected disease of worldwide importance, whose main control strategies are based on the diagnosis of sick individuals and their treatment. For this purpose, several diagnostic techniques are constantly being developed; however, they do not always have the necessary accuracy for this task. Molecular methods have proved to be sensitive and specific diagnostic tools for numerous conditions, including the infection of neglected tropical diseases (LV et al., 2022). Therefore, it can be the future solution for schistosomiasis's control.

Of the studies that used stool samples, studies 6 and 13 showed the highest molecular sensitivity. The first performed a cross-sectional study on a population (n = 297) of school-aged children (7 – 16 years) in two districts of Tanzania and aimed to compare the efficiency of POC-CCA and microscopy with a qPCR reaction. Although the molecular method showed high sensitivity (99.5%), when compared to Kato-Katz (KK), its specificity (29.55%) was the lowest among the studies that used a qPCR and the lowest among all the studies that used stool samples. However, employing a latent class analysis, the sensitivity of the molecular method have decreased from 99.5% to 98.7%, and the specificity increased to 81.2%. This may have resulted from the low sensitivity of KK, which has already been reported in the literature, reducing the specificity of the molecular method tested (Fuss et al., 2018; Meurs et al., 2017). Study 13, on the other hand, aimed to evaluate the occurrence of *S. mansoni* infection in an area of low endemicity in Minas Gerais, Brazil, using conventional PCR (n = 219). In this work, the sensitivity of PCR was considerably higher when compared with two KK slides (98.7% and 20.80%, respectively). In addition to the higher values of specificity and sensitivity, compared with KK, study 13 showed high predictive values (PPV = 100% and NPV = 99.30%, respectively), which lends credibility to the test (Monaghan et al., 2021; Altman & Blond, 1994). Therefore, it is noticed that in all the situations presented

above, the sensitivity of the molecular test was greater than the gold standard parasitological test, Kato-Katz, which demonstrates that KK alone may not be capable of correctly identifying infected individuals.

All studies that evaluated stool samples using LAMP, qPCR, PCR-ELISA, and conventional PCR resulted in a higher prevalence by the molecular methods than by the parasitological methods, which is shown by the ratio between prevalences, predictive values, sensitivity, and specificity of the tests discussed in our work, except study 23, which showed a higher prevalence for the parasitological method. This fact is due to the greater ability of molecular tests to identify true positives and true negatives, because of their potential to detect the presence of the parasite's DNA in the biological samples when compared with parasitological methods (Schwarz et al., 2014; Gandasegui et al., 2018; Senra et al., 2018). Thus, the use of molecular tests makes it possible to identify the true prevalence of the disease, which has been underestimated by the KK due to its low sensitivity.

Conventional PCR assays for urine samples proved to be very sensitive when compared with qPCR for the same type of sample. As described by study 5 and study 23, qPCR had the lowest sensitivity amongst other diagnostic methods applied in the research, including KK. The poor performance of the technique may be related to the inadequate conservation of the DNA extracted from urine samples (Fuss, Mazigo & Mueller, 2020). Other authors have reported better results using different methods of conservation and concentration, such as filtration, of the samples used in their assays, but it showed no improvement in the test result (Lodh et al., 2014; Anyan et al., 2020). Comparing Real-Time PCR to Conventional PCR, study 5 showed better molecular sensitivity results for Real Time PCR than the conventional PCR performed in study 15 and 23, which aimed to evaluate the PCR performance compared with other diagnostic methods in a low transmission community, using serum samples, urine, and stools. Techniques that rely on the detection of free DNA in urine samples have limitations regarding the concentration

of DNA in this kind of sample since the amount of DNA in this biological fluid is not always enough to determine the infection status (Umansky et al., 2006; Chen et al., 2019). Therefore, in the case of schistosomiasis mansoni, urine samples are not as suitable for identifying the disease.

Obtaining good quality DNA is essential for the confidence in the subsequent amplification steps. The extraction method can influence the assay, as observed in study 18 since if the DNA lacks adequate integrity and purity, it can interfere in the amplification process, which can decrease the sensitivity and specificity of the molecular method and, in some cases, inhibit amplification (Demeke, Jenkins, 2010).

Still regarding the studies that aimed to evaluate molecular tests in urine samples, study 18 obtained high sensitivity and specificity results using a LAMP reaction, depending on the extraction method used. In the literature, LAMP is an extremely explored methodology for its practicality (Li et al., 2017; Hu et al., 2020). As already mentioned, the extraction method used can influence the amplification result, as happened in study 18, which showed a greater sensitivity when using the QIAmp DNeasy Blood and Tissue Kit. This may be due to this kit providing a purer and more intact DNA sample than the other methods used (Santos et al., 2018). Unlike study 18, the study 23 that evaluated LAMP method and used the QIAmp DNA Blood Mini kit didn't presented a good sensitivity. Therefore, it is important to evaluate whether the DNA extraction method to be used will be suitable for the type of sample from which the DNA will be extracted, since a good extraction method must provide pure DNA without contaminants and without degradation.

Except in the examples cited above, the sensitivity of molecular methods was always higher than that of parasitological methods used and already described in the literature (Enk et al., 2012).

Of the 3 serum studies that proposed to develop a schistosomiasis mansoni molecular diagnostic methodology, study 5 obtained high sensitivity values. Whereas studies 5 and 21 obtained 100% values of molecular specificity. The

ratios between prevalence values were greater than 1 in all cases that brought prevalence results (3,5,6). The PPV values calculated by work 3 were low, while the NPV for the same work almost reached its maximum value. The NPV of study 5, which was the only predictive value presented, was high. The PPV values of study 21 were maximum for both real time PCR evaluated. The positive and negative predictive values are variables directly influenced by the sensitivities, specificities, and prevalence of the tests in a population, whose results can be explained both by the population characteristics and size, where the studies were conducted, and by factors intrinsic to the evaluated tests (Monaghan et al., 2021; Altman & Bland, 1994). Thus, in the same way as urine samples, serum samples do not have a concentration of DNA that is enough to the diagnosis of schistosomiasis mansoni, especially in matters of low parasitic load, due to this they tend to present a lower sensitivity when evaluated by molecular methods.

Molecular targets correspond to the regions of the *S. mansoni* genome that will be detected by the enzymes that amplify the DNA, and the success of the amplification is directly related to the extraction method, which can also influence the sensitivity of the test. The molecular targets used by the studies were the Sm1-7 region, ITS2 region, and mitochondrial minisatellite region (MIT) of *S. mansoni* (Hamburger et al., 1991; Obeng et al., 2008; Pena et al., 1995). In all studies, the molecular targets were amplified without significant differences in the results of sensitivity and specificity of the works regarding the target regions. Thus, despite being different, the molecular regions used for amplification in the methods of the 23 studies evaluated amplified and no differences in efficiency were observed in the methods due to the different targets.

5 CONCLUSIONS

The molecular diagnoses of schistosomiasis mansoni are excellent alternatives for schistosomiasis control. Stool samples, besides being more studied, showed high

sensitivity for different regions with different levels of endemicity and transmission, as well as urine samples. Serum samples were the least studied and showed the lowest sensitivity. Therefore, this work demonstrates the need for further research on the molecular diagnosis of schistosomiasis mansoni, to solve the major problem of the low accuracy gold standard, especially in areas of low endemicity for the disease.

ACKNOWLEDGEMENTS

The authors would like to thank the Aggeu Magalhães Institute for provide the infrastructure and training human resources to develop research. We are also thankful to Fiocruz and National Council for Scientific and Technological Development for granting scientific initiation scholarships to undergraduate students. This study was funded by National Council for Scientific and Technological Development – CNPq (PROEP/IAM: 400755/2019-0).

REFERENCES

Abbasi, I., King, C. H., Muchiri, E. M., & Hamburger, J. (2010). Detection of *Schistosoma mansoni* and *Schistosoma haematobium* DNA by loop-mediated isothermal amplification: identification of infected snails from early prepatency. *The American journal of tropical medicine and hygiene*, *83*(2), 427.

Altman, D. G., & Bland, J. M. (1994). Statistics Notes: Diagnostic tests 2: predictive values. *Bmj*, *309*(6947), 102.

Anyan, W. K., Pulkkila, B. R., Dyra, C. E., Price, M., Naples, J. M., Quartey, J. K., ... & Lodh, N. (2020). Assessment of dual schistosome infection prevalence from urine in an endemic community of Ghana by molecular diagnostic approach. *Parasite epidemiology and control*, *9*, e00130.

Bruscky, I. S., de Melo, F. L., de Medeiros, Z. M., Albuquerque, F. F., Wanderley, L. B., & da Cunha-Correia, C. (2016). Nested polymerase chain reaction in cerebrospinal fluid for diagnosing spinal cord schistosomiasis: A promising method. *Journal of the neurological sciences*, *366*, 87-90.

Carvalho, G. C. D., Marques, L. H. D. S., Gomes, L. I., Rabello, A., Ribeiro, L. C., Scopel, K. K. G., ... & Abramo, C. (2012). Polymerase chain reaction for the evaluation of *Schistosoma mansoni* infection in two low endemicity areas of Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz*, *107*, 899-902.

Cavalcanti, M. G., Cunha, A. F. A., & Peralta, J. M. (2019). The advances in molecular and new point-of-care (POC) diagnosis of schistosomiasis pre-and post-praziquantel use: in the pursuit of more reliable approaches for low endemic and non-endemic areas. *Frontiers in Immunology*, *10*, 858.

Casavechia, M. T. G., de Melo, G. D. A. N., Fernandes, A. C. B. D. S., De Castro, K. R., Pedroso, R. B., Santos, T. D. S., & Teixeira, J. J. V. (2018). Systematic review and meta-analysis on *Schistosoma mansoni* infection prevalence, and associated risk factors in Brazil. *Parasitology*, *145*(8), 1000-1014.

Chen, W., Liao, Y., Yang, C., Fang, Z., Liu, B., Zheng, X., & Zhou, C. (2019). Potential use of transrenal DNA for non-invasive monitoring and prognosis of colorectal cancer. *Biomarkers*, *24*(6), 524-529.

Demeke, T., & Jenkins, G. R. (2010). Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits. *Analytical and bioanalytical chemistry*, *396*, 1977-1990.

de Souza Gomes, E. C., Júnior, W. L. B., & de Melo, F. L. (2022). Evaluation of SmITS1-LAMP performance to diagnosis schistosomiasis in human stool samples from an endemic area in Brazil. *Experimental Parasitology*, *242*, 108389.

do Carmo Magalhães, F., Resende, S. D., Senra, C., Graeff-Teixeira, C., Enk, M. J., Coelho, P. M. Z., ... & Carneiro, M. (2020). Accuracy of real-time polymerase chain reaction to detect *Schistosoma mansoni*–infected individuals from an endemic area with low parasite loads. *Parasitology*, *147*(10), 1140-1148.

Enk, M. J., Oliveira e Silva, G., & Rodrigues, N. B. (2012). Diagnostic accuracy and applicability of a PCR system for the detection of *Schistosoma mansoni* DNA in human urine samples from an endemic area. *PloS one*, *7*(6), e38947.

Espírito-Santo, M. C. C., Alvarado-Mora, M. V., Dias-Neto, E., Botelho-Lima, L. S., Moreira, J. P., Amorim, M., ... & Gryschek, R. C. B. (2014). Evaluation of real-time PCR assay to detect *Schistosoma mansoni* infections in a low endemic setting. *BMC Infectious Diseases*, *14*, 1-10.

Ferrer, E., Villegas, B., Mughini-Gras, L., Hernández, D., Jiménez, V., Catalano, E., & Incani, R. N. (2020). Diagnostic performance of parasitological, immunological and molecular tests for the diagnosis of *Schistosoma mansoni* infection in a community of low transmission in Venezuela. *Acta tropica*, *204*, 105360.

Fuss, A., Mazigo, H. D., Tappe, D., Kasang, C., & Mueller, A. (2018). Comparison of sensitivity and specificity of three diagnostic tests to detect *Schistosoma mansoni* infections in school children in Mwanza region, Tanzania. *PloS one*, *13*(8), e0202499.

Fuss, A., Deogratias Mazigo, H., & Mueller, A. (2020). Evaluation of serum-based real-time PCR to detect *Schistosoma mansoni* infection before and after treatment. *Infectious Diseases of Poverty*, *9*(03), 114-120.

Gandasegui, J., Fernández-Soto, P., Muro, A., Simões Barbosa, C., Lopes de Melo, F., Loyo, R., & de Souza Gomes, E. C. (2018). A field survey using LAMP assay for detection of *Schistosoma mansoni* in a low-transmission area of schistosomiasis in Umbuzeiro, Brazil: Assessment in human and snail samples. *PLoS Neglected Tropical Diseases*, *12*(3), e0006314.

Hamburger, J., Turetski, T., Kapeller, I., & Deresiewicz, R. (1991). Highly repeated short DNA sequences in the genome of *Schistosoma mansoni* recognized by a species-specific probe. *Molecular and biochemical parasitology*, *44*(1), 73-80.

Hessler, M. J., Cyrs, A., Krenzke, S. C., Mahmoud, E. S., Sikasunge, C., Mwansa, J., & Lodh, N. (2017). Detection of duo-schistosome infection from filtered urine samples from school children in Zambia after MDA. *PLoS One*, *12*(12), e0189400.

Hoekstra, P. T., Madinga, J., Lutumba, P., van Grootveld, R., Brienen, E. A., Corstjens, P. L., ... & van Lieshout, L. (2022). Diagnosis of schistosomiasis without a microscope: evaluating circulating antigen (CCA, CAA) and DNA detection methods on banked samples of a community-based survey from DR congo. *Tropical Medicine and Infectious Disease*, *7*(10), 315.

Hoekstra, P. T., Chernet, A., de Dood, C. J., Brienen, E. A., Corstjens, P. L., Labhardt, N. D., ... & van Lieshout, L. (2022). Sensitive diagnosis and post-treatment follow-up of *Schistosoma mansoni* infections in asymptomatic Eritrean refugees by circulating anodic antigen detection and polymerase chain reaction. *The American journal of tropical medicine and hygiene*, *106*(4), 1240.

Hu, X., Deng, Q., Li, J., Chen, J., Wang, Z., Zhang, X., ... & Hou, T. (2020). Development and clinical application of a rapid and sensitive loop-mediated isothermal amplification test for SARS-CoV-2 infection. *MSphere*, *5*(4), e00808-20.

Katz, N. (2018). Inquérito Nacional de Prevalência da Esquistossomose mansoni e Geo-helmintoses.

Katz, N., Chaves, A., & Pellegrino, J. (1972). A simple, device for quantita tive stool thick-smear technique in schistosomiasis mansoni. *Revista do instituto de medicina tropical de São Paulo*, *14*(6), 397-400.

Lau, R., Makhani, L., Omoruna, O., Lecce, C., Shao, E., Cunanan, M., ... & Boggild, A. K. (2023). Performance characteristics of diagnostic assays for schistosomiasis in Ontario, Canada. *Therapeutic Advances in Infectious Disease*, *10*, 20499361231173843.

Li, Y., Fan, P., Zhou, S., & Zhang, L. (2017). Loop-mediated isothermal amplification (LAMP): A novel rapid detection platform for pathogens. *Microbial pathogenesis*, *107*, 54-61.

Lin, D. D., Xu, J. M., Zhang, Y. Y., Liu, Y. M., Hu, F., Xu, X. L., ... & Wu, G. L. (2008). Evaluation of IgG-ELISA for the diagnosis of Schistosoma japonicum in a high prevalence, low intensity endemic area of China. *Acta Tropica*, *107*(2), 128-133.

Lv, C., Deng, W., Wang, L., Qin, Z., Zhou, X., & Xu, J. (2022). Molecular techniques as alternatives of diagnostic tools in China as schistosomiasis moving towards elimination. *Pathogens*, *11*(3), 287.

Lodh, N., Naples, J. M., Bosompem, K. M., Quartey, J., & Shiff, C. J. (2014). Detection of parasite-specific DNA in urine sediment obtained by filtration differentiates between single and mixed infections of *Schistosoma mansoni* and *S. haematobium* from endemic areas in Ghana. *PLoS One*, *9*(3), e91144.

Lodh, N. (2013). Diagnosis of *Schistosoma mansoni* without the Stool: Comparison of Three Diagnostic Tests to Detect *Schiostosoma mansoni* Infection from Filtered Urine in Zambia (vol 89, pg 46, 2013). *American Journal of Tropical Medicine and Hygiene*, *89*(3), 608-608.

Mastella, J. O. (2015). Análise de classes latentes: da teoria à prática.

Mesquita, S. G., Caldeira, R. L., Favre, T. C., Massara, C. L., Beck, L. C. N. H., Simões, T. C., ... & Fonseca, C. T. (2022). Assessment of the accuracy of 11 different diagnostic tests for the detection of Schistosomiasis mansoni in individuals from a Brazilian area of low endemicity using latent class analysis. *Frontiers in Microbiology*, *13*, 1048457.

Meurs, L., Polderman, A. M., Vinkeles Melchers, N. V., Brienen, E. A., Verweij, J. J., Groosjohan, B., ... & van Lieshout, L. (2017). Diagnosing polyparasitism in a high-prevalence setting in Beira, Mozambique: detection of intestinal parasites in fecal samples by microscopy and real-time PCR. *PLoS neglected tropical diseases*, *11*(1), e0005310.

Monaghan, T. F., Rahman, S. N., Agudelo, C. W., Wein, A. J., Lazar, J. M., Everaert, K., & Dmochowski, R. R. (2021). Foundational statistical principles in medical research: sensitivity, specificity, positive predictive value, and negative predictive value. *Medicina*, *57*(5), 503.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic acids research*, *28*(12), e63-e63.

Noya, O., Katz, N., Pointier, J. P., Theron, A., & de Noya, B. A. (2015). Schistosomiasis in America. *Neglected tropical diseases-Latin America and the Caribbean*, 11-43.

Obeng, B. B., Aryeetey, Y. A., De Dood, C. J., Amoah, A. S., Larbi, I. A., Deelder, A. M., ... & Van Lieshout, L. (2008). Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of Schistosoma haematobium in urine samples from Ghana. *Annals of Tropical Medicine & Parasitology*, *102*(7), 625-633.

Pellon, B., & Teixeira, I. (1950). Distribuição geográfica da esquistossomose mansônica no Brasil. In *Distribuição Geografica da Esquistossomose Mansonica no Brasil* (pp. 203-203).

Pena, H. B., de Souza, C. P., Simpson, A. J., & Pena, S. D. (1995). Intracellular promiscuity in *Schistosoma mansoni*: nuclear transcribed DNA sequences are part of a mitochondrial minisatellite region. *Proceedings of the National Academy of Sciences*, *92*(3), 915-919.

Pontes, L. A., Oliveira, M. C., Katz, N., Dias-Neto, E., & Rabello, A. N. A. (2003). Comparison of a polymerase chain reaction and the Kato-Katz technique for diagnosing infection with *Schistosoma mansoni*. *The American journal of tropical medicine and hygiene*, *68*(6), 652-656.

Pontes, L. A., Dias-Neto, E., & Rabello, A. (2002). Detection by polymerase chain reaction of *Schistosoma mansoni* DNA in human serum and feces. *The American journal of tropical medicine and hygiene*, *66*(2), 157-162.

Price, M., Cyrs, A., Sikasunge, C. S., Mwansa, J., & Lodh, N. (2019). Testing the infection prevalence of *Schistosoma mansoni* after mass drug administration by comparing sensitivity and specificity of species-specific repeat fragment amplification by PCR and loop-mediated isothermal amplification. *The American Journal of Tropical Medicine and Hygiene*, *101*(1), 78.

Sandoval, N., Siles-Lucas, M., Aban, J. L., Pérez-Arellano, J. L., Gárate, T., & Muro, A. (2006). *Schistosoma mansoni*: a diagnostic approach to detect acute schistosomiasis infection in a murine model by PCR. *Experimental parasitology*, *114*(2), 84-88.

Santos, A. L. F., Oliveira, C. Q., Arruda, G. N. P., & Martins, J. K. (2018). Comparison of DNA extraction using proteinase K and extraction kit: analysis of the quality of the genetic material. *Jornal Brasileiro de Patologia e Medicina Laboratorial*, *54*, 70-75.

Senra, C., Gomes, L. I., Siqueira, L. M. V., Coelho, P. M. Z., Rabello, A., & Oliveira, E. (2018). Development of a laboratorial platform for diagnosis of schistosomiasis mansoni by PCR-ELISA. *BMC Research Notes*, *11*, 1-5.

Schunk, M., Kebede Mekonnen, S., Wondafrash, B., Mengele, C., Fleischmann, E., Herbinger, K. H., ... & Zeynudin, A. (2015). Use of occult blood detection cards for real-time PCR-based diagnosis of *Schistosoma mansoni* infection. *PLoS One*, *10*(9), e0137730.

Schwarz, N. G., Rakotozandrindrainy, R., Heriniaina, J. N., Randriamampionona, N., Hahn, A., Hogan, B., ... & Hagen, R. M. (2014). *Schistosoma mansoni* in schoolchildren in a Madagascan highland school assessed by PCR and sedimentation microscopy and Bayesian estimation of sensitivities and specificities. *Acta tropica*, *134*, 89-94.

Sue, M. J., Yeap, S. K., Omar, A. R., & Tan, S. W. (2014). Application of PCR-ELISA in molecular diagnosis. *BioMed research international*, *2014*.

Umansky, S. R., & Tomei, L. D. (2006). Transrenal DNA testing: progress and perspectives. *Expert review of molecular diagnostics*, *6*(2), 153-163.

Weerakoon, K. G., Gobert, G. N., Cai, P., & McManus, D. P. (2015). Advances in the diagnosis of human schistosomiasis. *Clinical microbiology reviews*, *28*(4), 939-967.

World Health Organization. (2016). Schistosomiasis and soil-transmitted helminthiases: number of people treated in 2015. *Weekly epidemiological record*, *91*(49/50), 585-595.

World Health Organization. (2021, May 18). Schistosomiasis. In Schistosomiasis. Retrieved December 3, 2021, from https://www.who.int/news-room/fact-sheets/detail/schistosomiasis

Xu, J., Peeling, R. W., Chen, J. X., Wu, X. H., Wu, Z. D., Wang, S. P., ... & Zhou, X. N. (2011). Evaluation of immunoassays for the diagnosis of Schistosoma japonicum infection using archived sera. *PLoS neglected tropical diseases*, *5*(1), e949.

Zhou, X. N., Xu, J., Chen, H. G., Wang, T. P., Huang, X. B., Lin, D. D., ... & Peeling, R. W. (2011). Tools to support policy decisions related to treatment strategies and surveillance of Schistosomiasis japonica towards elimination. *PLoS neglected tropical diseases*, *5*(12), e1408.

Authorship contributions

1 - Eduardo Henrique Matos Pires

Bachelor in Biological Sciences by the Universidade Federal Rural de Pernambuco (UFRPE). Currently a Masters's student of the Programa de Pós-graduação em Biociências e Biotecnologia em Saúde (PPGBBS) by the Instituto Aggeu Magalhães/FIOCRUZ-PE.
https://orcid.org/0000-0001-8354-3227 • dudu.matos@outlook.com
Contribution: Conceptualization, Methodology, Data Curation, Writing – First Writing and Editing, Data Visualization.

2 - Natália Adriely Ribeiro da Silva Costa

Graduate in Biomedicine at the Universidade Federal de Pernambuco (UFPE). Currently a PIBIC-FIOCRUZ student working in schistossomiasis's diagnostic.
https://orcid.org/0000-0001-7256-3054 • adriely11@live.com
Contribution: Conceptualization, Methodology, Data Curation, Writing – First Writing and Editing, Data Visualization.

3 - Wheverton Ricardo Correia do Nascimento

Adjunct professor of the Academic Area of Tropical Medicine and Permanent member of the Postgraduate Program in Morphotechnology (PPGM) at Universidade Federal de Pernambuco
https://orcid.org/0000-0003-0434-4831 • wheverton.nascimento@fiocruz.br
Contribution: Writing – Review and Editing, Supervision.

4 - Walter Lins Barbosa Junior

PhD, and postdoc in Sciences by the Programa de Pós-graduação em Biociências e Biotecnologia em Saúde (PPGBBS) at the Instituto Aggeu Magalhães/FIOCRUZ-PE. Currently Senior Lab Analyst of the Laboratório de Referência em Esquistossomose (LRE) at the Instituto Aggeu Magalhães/FIOCRUZ-PE.
https://orcid.org/0000-0002-8301-6600 • walter.lins@fiocruz.br
Contribution: Writing – Review and Editing, Supervision.

5 - Elainne Christine de Souza Gomes

Researcher in Public Health - IAM/ Fiocruz/ PE (Department of Parasitology) and Coordinator of the Laboratório de Referência em Esquistossomose (LRE)
https://orcid.org/0000-0001-7836-6457 • elainne.gomes@fiocruz.br
Contribution: Conceptualization, Writing – Review and Editing, Supervision, Project Administration

How to quote this article

Pires, E. H. M., Costa, N. A. R. da S., Nascimento, W. R. C. do, Barbosa Júnior, W. L., & Gomes, E. C. de S. (2024). Molecular diagnosis of schistosomiasis mansoni: a literature review. *Ciência e Natura*, 46, e69398. https://doi.org/10.5902/2179460X69398